Loss of Rap1GAP in Papillary Thyroid Cancer

Anoma Nellore, Karolina Paziana, Changqing Ma, Oxana M. Tsygankova, Yan Wang, Kanchan Puttaswamy, Ammarah U. Iqbal, Susanna R. Franks, Yu Lv, Andrea B. Troxel, Michael D. Feldman, Judy L. Meinkoth, and Marcia S. Brose

Departments of Medicine (A.N., M.S.B.), Otorhinolaryngology: Head and Neck Surgery (K.Pa., C.M., Y.W., K.Pu., A.U.I., S.R.F., M.S.B.), Pharmacology (O.M.T., J.L.M.), Biostatistics and Epidemiology (A.B.T.) and Pathology (M.D.F.), University of Pennsylvania, Philadelphia, Pennsylvania 19104; and Department of Pathology (Y.L.), Capital University of Medical Sciences, 100069 Beijing, China

Context: Rap1 GTPase-activating protein (GAP) regulates the activity of Rap1, a putative oncogene. We previously reported Rap1GAP was highly expressed in normal human thyroid cells and decreased in five papillary thyroid carcinomas (PTCs).

Objectives: To confirm the significance of these findings, we analyzed Rap1GAP expression in a larger set of benign tumors (adenomas and hyperplastic nodules) and PTCs. We determined whether the presence of the BRAFV600E mutation or allelic loss of Rap1GAP related to changes in Rap1GAP protein expression. To determine the consequences of Rap1GAP loss, we targeted Rap1GAP in culture using small interfering RNA.

Design, Patients, and Methods: A highly specific Rap1GAP antibody was applied to sections of 55 human thyroid tissues. Genomic DNA was analyzed for the presence of the BRAFV600E mutation, and loss of Rap1GAP. Rap1GAP expression in rat thyroid cells was abolished using small interfering RNA.

Results: We observed that down-regulation of Rap1GAP in benign lesions and PTCs was common. Rap1GAP expression was more severely decreased in PTCs. Loss of Rap1GAP expression was observed in multiple histological variants of PTCs. Approximately 20% of PTCs and adenomas exhibited allelic loss of Rap1GAP. Loss of Rap1GAP was not associated with the presence of the BRAFV600E mutation. In vitro, loss of Rap1GAP was sufficient to increase Rap1 activity in thyroid cells.

Conclusions: These data indicate that loss of Rap1GAP is a frequent event in PTC. The more frequent and greater down-regulation of Rap1GAP in PTCs compared with adenomas suggests a role for Rap1GAP depletion in the progression of human thyroid tumors, possibly through unrestrained Rap activity. (J Clin Endocrinol Metab 94: 1026–1032, 2009)
ability to inhibit the proliferation of rat thyroid cells when over-expressed (5). Since then, decreased expression of Rap1GAP in pancreatic carcinomas has been reported (6).

Although widely used as a tool to inhibit Rap activity, little is known about the biological function and regulation of cellular Rap1GAP. Our previous work revealed that Rap1GAP is abundant in differentiated rat thyroid epithelial cells and that TSH regulates Rap1GAP protein stability (5). More recently we reported that Rap1GAP expression is decreased in human thyroid carcinoma-derived cell lines that had undergone epithelial-to-mesenchymal transition. Restoring Rap1GAP expression to these cells impaired cell migration, invasion, and anchorage-independent proliferation (7). To confirm the physiological significance of these findings, we analyzed Rap1GAP expression in primary thyroid tumors. As we predicted, Rap1GAP was highly expressed in normal thyroid follicular cells and its expression markedly decreased in papillary thyroid tumors (PTCs) (7).

In this study, we set out to determine the clinical significance of these findings by examining Rap1GAP staining in a larger number of human thyroid tumors. This analysis revealed that the expression of Rap1GAP is decreased in the overwhelming majority of PTCs. Rap1GAP was decreased in multiple histological variants of PTC, suggesting that Rap1GAP down-regulation is a frequent event. Strikingly, Rap1GAP expression was retained in approximately 50% of the benign adenomas analyzed. In contrast to adenomas in which Rap1GAP expression was modestly diminished in some samples, PTCs had a greater loss of Rap1GAP expression in the vast majority of samples. These data raise the interesting possibility that depletion of Rap1GAP contributes to thyroid tumorigenesis.

**Materials and Methods**

**Transfection with small interfering RNAs (siRNAs)**

Wistar rat thyroid (WRT) cells were propagated as described previously (7). Rap1GAP-directed and scrambled siRNAs were introduced into WRT cells using the Amaxa Nucleofector (Koeln, Germany) according to the manufacturer’s recommendations. Cells (1 × 10⁶) were trypsinized and subjected to electroporation in suspension in the presence of 100–200 nM scrambled siRNA (1027280) and Rap1GAP duplexes (SI01737043, SI01737050) purchased from QIAGEN (Valencia, CA). The cells were plated overnight and Rap1 activation assessed at 48 h after transfection.

**Rap1 activation**

A glutathione-S-transferase fusion of the Rap binding domain of RaLGDS was expressed and purified as described previously (8). Rap1 activation was assessed essentially as described in (9) with minor modifications. Cells were grown to 80% confluence and starved overnight. After lysis in modified radioimmunoprecipitation assay buffer [50 mM Tris-HCl (pH 8.0), 150 mM NaCl, 0.5% deoxycholate, 1% Nonidet P-40, 0.1% sodium dodecyl sulfate, 10 mM NaF, 2 mM Na₃VO₄, aprotonin, leupeptin, pefabloc each 10 μg/ml] containing 50 μg/ml glutathione-S-transferase-RaLGDS Rap binding domain, protein concentrations were determined using DC protein assay (Bio-Rad Laboratories, Hercules, CA). Cell proteins (400 μg) were incubated with glutathione-Sepharose beads for 1 h at 4°C. After three washes in lysis buffer, bound proteins were eluted in sample buffer, heated for 5 min at 95°C, separated on 12% SDS-PAGE, and subjected to immunoblotting. In parallel, total cell lysates (20 μg) were analyzed by Western blot with a polyclonal Rap1-specific antibody.

**Immunohistochemistry**

Tissue blocks from patients diagnosed with papillary thyroid carcinoma were provided by Dr. Yu Lv (Department of Pathology, Beijing Chaoyang Hospital, Capital University of Medical Sciences, Beijing, China). Blocks were chosen on the basis of containing both tumor tissue and adjacent normal within the same block (and sections). Fresh hematoxylin and eosin sections were made and reviewed by trained pathologists at the Hospital of the University of Pennsylvania. Fresh-cut 5-μm sections were used for immunohistochemical staining. Sections were incubated at 58°C for 20 min, deparaffinized in xylene 2 × 15 min, and rehydrated. Sections were incubated in antigen-unmasking solution (Vector Laboratories, Burlingame, CA) at 95–98°C for 20 min, cooled to room temperature, and endogenous peroxidase activity blocked by incubation in 3% H₂O₂ at room temperature for 15 min. Sections were washed in PBS-Tween 20, incubated in blocking buffer (10% normal goat serum, 1% BSA in PBS-Tween 20), and incubated with primary antibody (Rap1GAP, 1:500; Santa Cruz Biotechnology, Santa Cruz, CA) at 4°C overnight. After washing, sections were stained with biotinylated goat antirabbit IgG (1:200) for 30 min at room temperature, washed, and incubated in ABC complex (Vector Vectastain Elite ABC kit) at room temperature for 30 min. Bound antibody was visualized by diaminobenzidine (Vector Laboratories). Sections were analyzed by two pathologists. All cases were evaluated morphologically. Immunohistochemical stains were scored on a 0–3 scale, with 3+ being strong, granular cytoplasmic staining, 2+, moderately intense staining, 1+, weak staining, and 0, no stain. Paired readings of staining intensity for both the tumor tissue and adjacent normal tissue in the same section were recorded.

**Statistical analysis**

All data are summarized in tabular and graphical form using means, medians, ranges, and sds for continuous or semicontinuous variables and using frequencies for binary variables. Differences in staining intensity between adjacent normal and tumor tissues were evaluated using the Wilcoxon signed-rank test (the nonparametric equivalent to the paired t test). To assess whether the within-patient difference in staining intensity varied by subgroups (e.g. PTC vs. benign), we applied Wilcoxon rank sum test. All tests were conducted using a two-sided α-level of 0.05.

**DNA isolation and sequencing**

Archival paraffin-embedded tissue blocks of PTCs and benign nodules were sectioned and marked for areas of greater than 70% tumor using hematoxylin and eosin-stained sections as references. The marked areas were macrodissected and genomic DNA was extracted using the QIAamp DNA minikit (QIAGEN). BRAF exon 15 was amplified via PCR using the following intron-based primers: BRAF exon 15 forward, 5′-ATGCTTGCTCTGATAGGAAAATGA-3′, BRAF exon 15 reverse, 5′-TGATT TTTGTGAATCTGGGAACCTATGA-3′. PCR was performed under the following conditions: initial denaturation at 95°C for 5 min, then denaturation at 95°C for 30 sec, annealing at 55°C for 30 sec and elongation at 72°C for 30 sec for 35 cycles, followed by a final extension at 72°C for 5 min. The final products were fractionated on a 1% agarose gel, and resulting bands were cut and extracted using the MiniElute gel extraction kit (QIAGEN). Samples were then sent for Sanger sequencing on an ABI (Applied Biosystems, Foster City, CA) 3100 capillary sequencer at the DNA Sequencing Facility at the Abramson Comprehensive Cancer Center at the University of Pennsylvania. BRAF sequencing results were successfully obtained from 53 of 57 samples. The quality of the remaining four DNA samples was insufficient to obtain reliable sequencing results.

**Allelic loss of Rap1GAP**

Copy number data were generated using primers for Rap1GAP intron 9. Quantitative real-time PCR (qPCR) was used for detecting copy
number changes of the Rap1GAP gene. Unless specified, all reagents were from Applied Biosystems. All PCR plates were run on an Applied Biosystems 7900HT fast-real-time PCR system. Primer Express software version 2.0 (Applied Biosystems) was used to design primer sets using sequencing of the intron 9 of the Rap1GAP gene. During primer design, frequently repeated sequences in the genome were excluded using RepeatMasker (http://www.repeatmasker.org/), and all primers and probes were thoroughly analyzed with a BLAT search (http://genome.ucsc.edu/) to prevent nonspecific priming. TATA-box binding protein (TBP; at 6q27) was used as the endogenous control gene and a set of primer-probe was designed for it as described above.

Primer-probe sets of the Rap1GAP and TBP genes were first tested for PCR efficiencies by running qPCR with the relative standard curve method. Because efficiencies were comparable between them and very close to 100%, relative quantification using the comparative cycle threshold (CT) method (ΔΔCT method) was applied for copy number analyses. Human genomic DNA extracted from whole blood from CLONTECH (Mountain View, CA; catalog no. 636401) was used as the calibrator diploid sample for qPCR.

Results were analyzed with Applied Biosystems 7900HT sequence detection system version 2.3 software using relative quantification study (ΔΔCT study) according to the manufacturer’s instructions. Copy numbers were reported as relative to TBP, which is diploid in most if not all tissues and expresses uniformly across all sample types. We used the threshold of relative quantification less than 0.8 to identify the presence of DNA copy number loss in our samples.

**Results**

**Rap1GAP expression is decreased in human PTCs**

We expanded our study of human PTCs from the initial five samples that we first studied to an additional set of 38 PTCs (Table 1). A highly specific Rap1GAP antibody (6, 7) was used to stain primary thyroid tumors. The Rap1GAP antibody recognized a 95-kDa protein doublet that corresponds to differentially phosphorylated forms of Rap1GAP (5). The specificity of the Rap1GAP antibody was further documented by showing that it detected HA-Rap1GAP expressed from an adenovirus in WRT cells (Fig. 1A). Additionally, depletion of Rap1GAP using siRNAs abolished Rap1GAP staining in immunostaining and Western blotting experiments (Fig. 1B and C). In all cases, Rap1GAP expression in tumor cells was compared with its expression in adjacent normal thyroid tissue in the same sections. Compared with adjacent normal tissue, the expression of Rap1GAP was decreased in PTCs (Fig. 1, A and B). As reported previously, Rap1GAP expression was unchanged in two of three hyperplastic nodules (Table 1). A highly specific Rap1GAP antibody (6, 7) was used to detect ectopic HA-Rap1GAP was examined. WRT cells (−) were infected with an adenovirus expressing HA-Rap1GAP (+) and harvested after 48 h. Total cell lysates were prepared and subjected to Western blotting with HA antibody. The same blot was reprobed with Rap1GAP antibody. The Rap1GAP antibody reacts with the same protein doublet as the HA antibody. B and C, HT29 colon carcinoma cells were transiently transfected with control (scrambled, scr) and Rap1GAP-directed siRNAs and subjected to immunostaining (B) and Western blotting (C) with the Rap1GAP antibody. Depletion of Rap1GAP abolished the signal in both assays.

**Loss of Rap1GAP in benign lesions**

To determine whether the decrease in Rap1GAP expression was specific to PTCs, Rap1GAP staining was examined in 19 benign lesions and compared with adjacent normal tissue in the same slide. This set of samples included 16 benign adenomatous nodules and three hyperplastic nodules (Table 1). In a significant proportion of benign nodules (nine of 19), Rap1GAP expression was decreased compared with adjacent normal tissue (Figs. 2 and 3). Strikingly, the decrease in Rap1GAP expression was far less pronounced in the benign lesions (see Fig. 2, C and D, for an example) compared with PTCs (Fig. 2A and B), although it was statistically significant (P < 0.0001). Down-regulation of Rap1GAP was an extremely frequent event in PTCs. Rap1GAP staining was decreased in 92% (35 of 38) of the PTCs examined (Fig. 2).

![Fig. 1. Expression of Rap1GAP protein in human tumors. A. To confirm the specificity of the Rap1GAP antibody, its ability to detect ectopic HA-Rap1GAP was examined. WRT cells (−) were infected with an adenovirus expressing HA-Rap1GAP (+) and harvested after 48 h. Total cell lysates were prepared and subjected to Western blotting with HA antibody. The same blot was reprobed with Rap1GAP antibody. The Rap1GAP antibody reacts with the same protein doublet as the HA antibody. B and C, HT29 colon carcinoma cells were transiently transfected with control (scrambled, scr) and Rap1GAP-directed siRNAs and subjected to immunostaining (B) and Western blotting (C) with the Rap1GAP antibody. Depletion of Rap1GAP abolished the signal in both assays.](https://academic.oup.com/jcem/article-abstract/94/3/1026/2596945/fig1.png)

**TABLE 1.** Summary of thyroid tissues analyzed for Rap1GAP protein expression and genotyped for BRAFV600E.

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>IHC (n)</th>
<th>Genotype (n)</th>
<th>V600E (%)</th>
<th>WT (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Papillary Thyroid Carcinoma</td>
<td>38</td>
<td>34</td>
<td>11 (52)</td>
<td>23 (68)</td>
</tr>
<tr>
<td>Classic</td>
<td>29</td>
<td>26</td>
<td>11 (42)</td>
<td>15 (58)</td>
</tr>
<tr>
<td>Follicular Variant</td>
<td>8</td>
<td>7</td>
<td>0 (0)</td>
<td>7 (100)</td>
</tr>
<tr>
<td>Tall Cell</td>
<td>1</td>
<td>1</td>
<td>0 (0)</td>
<td>1 (100)</td>
</tr>
<tr>
<td>Adenoma/Adenomatous Nodule</td>
<td>16</td>
<td>16</td>
<td>0 (0)</td>
<td>16 (100)</td>
</tr>
<tr>
<td>Hyperplastic Nodule</td>
<td>3</td>
<td>3</td>
<td>0 (0)</td>
<td>3 (100)</td>
</tr>
<tr>
<td>Total</td>
<td>57</td>
<td>53</td>
<td>11 (21)</td>
<td>42 (79)</td>
</tr>
</tbody>
</table>

IHC, Immunohistochemistry; WT, wild type.
The decrease in Rap1GAP expression is significantly greater in carcinomas vs. benign nodules (adenomatous or hyperplastic)

The mean Rap1GAP staining intensity of normal tissue (2.4 ± 0.5), benign nodules (2.18 ± 0.65), and PTCs (1.15 ± 0.52) are shown in Fig. 4A. The magnitude of the loss of Rap1GAP staining was 0.42 ± 0.12 for benign tumors and 1.15 ± 0.11 for papillary thyroid cancer when compared with adjacent normal tissue on the same slide (Fig. 4B). In both the carcinoma and benign thyroid tissues, the staining intensity was less in the nodule than in the adjacent normal tissue (P < 0.001 and P < 0.002, respectively). This analysis revealed that Rap1GAP expression was significantly decreased in both benign lesions and carcinomas compared with adjacent normal tissue. To determine whether the decrease in Rap1GAP expression was statistically different between PTCs and benign lesions, we performed a Wilcoxon rank sum test on the expression observed in each tumor-normal pair. This analysis revealed that the extent of Rap1GAP down-regulation was significantly greater in PTCs than in benign lesions (P < 0.0001).

Decrease in Rap1GAP staining is not associated with BRAFV600E genotype

Rap is the best-characterized activator of B-Raf (11). Therefore, we investigated whether the decrease in Rap1GAP expression was related to the presence of the BRAFV600E mutation. All tissue was genotyped for the BRAFV600E allele using Sanger sequencing (Table 1). We detected the BRAFV600E allele in 11 of 34 PTCs, a frequency that is consistent with prior reports (12–15). As expected, no mutations were detected in the benign nodules. Next we determined whether the presence of the BRAFV600E allele affected the loss of Rap1GAP staining observed in the PTCs. We detected no difference in the magnitude of the loss of Rap1GAP staining in samples that harbored the BRAFV600E allele compared with those with wild-type B Raf (Figs. 3 and 4C). Therefore, down-regulation of Rap1GAP was not preferentially associated with wild-type B Raf, suggesting that depletion of Rap1GAP is an independent event.

Decrease in Rap1GAP staining is associated with allelic loss in PTC

To identify potential causes for decreased expression of Rap1GAP in PTC and benign thyroid nodules, we used qPCR to detect changes in copy number of Rap1GAP in samples in which we had available DNA. Using this method, we identified loss of one copy of Rap1GAP in seven of 36 PTCs (19%), four of which also harbored the BRAFV600E mutation, and four of 19 benign nodules (21%), all of which were BRAFwt. Thus, whereas copy number loss likely contributes to loss of expression of Rap1GAP in these samples, it is not the only mechanism for decreased expression of Rap1GAP.

Decreased expression of Rap1GAP is sufficient to activate Rap1

To assess whether down-regulation of Rap1GAP elicited functional consequences, the effects of depleting Rap1GAP expression from rat thyroid follicular cells was examined. Acute down-regulation of Rap1GAP expression using RNA interference was sufficient to increase Rap1 activity in the absence of exogenous stimulus (Fig. 5). These results indicate that decreases in Rap1GAP expression are sufficient to increase Rap1 activity.

Discussion

Emerging evidence implicates a role for Rap1GAP, an essential negative regulator of cellular Rap proteins, as a tumor suppressor. This notion is clearly supported by data showing that stable overexpression of Rap1GAP in squamous cell carcinoma or pancreatic adenocarcinoma cell lines impaired tumor formation in mouse models (6, 16). Little is known regarding the contribution of Rap1GAP to malignant transformation in human tumors. Molecular profiling revealed a decrease in Rap1GAP expression in pancreatic cancer (17). Immunohistochemical staining revealed that Rap1GAP expression was decreased in a small proportion of in situ pancreatic adenocarcinomas and in a much greater percentage of poorly differentiated pancreatic adenocarcinomas (6).

Signaling in thyroid cancer has been recently shown to have significant clinical implications. Mutations in B Raf, in particular BRAFV600E, occur with high prevalence in PTCs (12–15). This mutation not only correlates with up-regulation of tumor-
igenic proteins, but it is also associated with thyroid tumor recurrence and invasion (18). Multikinase inhibitors, which target activated vascular endothelial growth factor receptor (19–21) and in one case RAF (18), are emerging as promising new treatments for metastatic papillary thyroid carcinoma. Given that cellular Rap proteins, the best-characterized targets of Rap1GAP, are potent activators of BRAF (11), loss of Rap1GAP activity may have clinical significance. In a preliminary analysis of five papillary thyroid carcinomas, Rap1GAP expression was found to be markedly reduced compared with adjacent normal thyroid tissue (7), prompting us to examine Rap1GAP expression in a larger set of human thyroid tumors.

Using immunohistochemical staining with a highly specific Rap1GAP antibody, we found Rap1GAP expression to be consistently and markedly decreased in a set of 38 papillary thyroid carcinomas ($P < 0.0001$). Compared with adjacent normal thyroid tissue, the expression of Rap1GAP was also decreased in benign lesions ($P < 0.004$), although not to the same extent as in carcinomas. The fact that the decrease observed in carcinomas was more frequent and greater than that seen in benign lesions ($P < 0.0001$) implies that decreases in Rap1GAP expression are associated with increased tumorigenicity. Taken together, these data provide compelling support for a role for Rap1GAP depletion in the evolution of thyroid cancer.

To explore potential mechanisms through which depletion of Rap1GAP contributes to tumor progression, Rap1GAP expression was acutely eliminated from thyroid cells using RNA interference. Strikingly, decreased expression of Rap1GAP was sufficient to increase Rap1 activity in the absence of exogenous stimuli. These findings indicate that Rap1GAP is an essential negative regulator of Rap1 activity and thyroid tumors that lack Rap1GAP are likely to exhibit enhanced Rap activity. In the presence of goitrogen (TSH), targeted expression of activated Rap1 to the thyroid gland in mice induced hyperplasia and, over time, invasive follicular cell carcinoma (22). The requirement for elevated TSH likely reflects the fact that Rap1 must be activated and phosphorylated to enhance cAMP-stimulated proliferation in rat thyroid cells (23). Hence, in benign lesions and primary differentiated thyroid tumors that retain TSH receptor expression (24), decreased expression of Rap1GAP might contribute to enhanced proliferation.

Rap1 is best known for its roles in the regulation of cell adhesion. Rap1 regulates inside-out signaling through integrins and is a target of outside-in signaling by cell-cell adhesion molecules, including E-cadherin (25). A role for Rap1 in the regulation of cell adhesion first emerged from genetic studies in Dro sophila in which Rap1 deficiency induced defects in the even distribution of drosophila E-cadherin (26). Recent studies revealed that activation (27) and inactivation of Rap1 (28, 29) are
required for the dynamic regulation of cell/cell contacts in epithelial cells. Hence, alterations in the balance of Rap activity would be expected to dysregulate cell/cell adhesion. Disruption of cell/cell contacts is associated with severe pathological consequences, including enhanced migratory and invasive properties. Intriguingly, Rap1GAP expression was selectively down-regulated in human thyroid carcinoma cell lines that no longer exhibited an epithelial morphology or expressed E-cadherin (7). Thyroid cancer cell lines that lacked Rap1GAP exhibited enhanced migratory and invasive properties compared with cell lines that retained Rap1GAP expression. Restoring Rap1GAP expression to deficient tumor cell lines impaired cell migration and invasion (7). Thus, down-regulation of Rap1GAP may facilitate the acquisition of invasive tumor behavior due to impaired cell/cell adhesion, an area of current investigation.

The molecular basis underlying the decrease in Rap1GAP expression in thyroid tumors is a subject of ongoing investigation. The Rap1GAP gene maps to 1p35-36 (30), a chromosomal region subject to deletion in human tumors (31, 32). Loss of heterozygosity at the Rap1GAP locus was reported in pancreatic adenocarcinoma (6). Although papillary thyroid cancer has been associated with relative copy number stability, we identified loss of one copy of Rap1GAP in 19% of PTCs and 21% of benign nodules. The molecular basis underlying decreased expression of Rap1GAP in the remaining tumors remains to be determined. In principle, Rap1GAP protein and/or message levels could be down-regulated. TSH regulates Rap1GAP protein stability in differentiated rat thyroid cells (5). It will be interesting to determine whether the molecular basis for decreased Rap1GAP staining is similar or different in tumors that retain expression of the TSH receptor vs. those that lost TSH regulation. Acute expression of activated Ras decreased Rap1GAP protein and message levels in rat thyroid cells and Rap1GAP message levels were down-regulated in thyroid cancer cell lines (7). Whether this reflects epigenetic silencing or mutational events that affect promoter activity or splicing remains to be determined.

The relationship between down-regulation of Rap1GAP and other mutational events in thyroid tumors remains to be explored in depth. Our preliminary findings indicate that there was not a significant correlation between down-regulation of Rap1GAP and the presence or absence of the BRAFV600E allele. Although we previously reported that acute expression of oncogenic Ras down-regulated Rap1GAP expression in rat thyroid cells (7), Ras mutations are infrequent in papillary thyroid carcinomas. It will be important to determine whether depletion of Rap1GAP is correlated with other mutational events and whether down-regulation of Rap1GAP is a frequent event in other subtypes of thyroid tumors. Further research into the mechanism of Rap1GAP loss and the mechanism through which
depletion of Rap1GAP contributes to tumor progression may identify novel targets for the development of other therapeutic strategies for the treatment of thyroid tumors. Already multi kinase inhibitors have shown activity in thyroid cancer. In summary, we have shown that progressive loss of Rap1GAP is a common feature in papillary thyroid cancer, and in so doing, our data imply that pathways regulated by Rap1GAP may harbor tractable therapeutic targets for this disease.

Acknowledgments

Address all correspondence and requests for reprints to: Marcia S. Brose, M.D., Ph.D., Department of Otorhinolaryngology: Head and Neck Surgery, Department of Medicine, Division of Hematology/Oncology, Abramson Comprehensive Cancer Center The University of Pennsylvania, Clinical Research Building, Room 127, 415 Curie Boulevard, Philadelphia, Pennsylvania 19104. E-mail: brosem@mail.med.upenn.edu.

This work was supported by Public Health Service Grants DK53757 and CA109543 (to J.L.M.). M.S.B. is a Damon Runyon-Siemens Clinical Investigator supported (in part) by the Damon Runyon Cancer Research Foundation (CI-25-05).

Current address for Y.W.: GlaxoSmithKline, King of Prussia, Pennsylvania.

References

12. de Rooy J, Bos JL 1997 Minimal Ras-binding domain of Rap1 can be used as an activation-specific probe for Ras. Oncogene 14:623–625
15. Xu X, Quiros RM, Gattuso P, Ain KB, Prinz RA 2003 High prevalence of BRAF gene mutation in papillary thyroid carcinomas and thyroid tumor cell lines. Cancer Res 63:4561–4567

Downloaded from https://academic.oup.com/jcem/article-abstract/94/3/1026/2596945 by guest on 16 April 2019